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Peripheral decrease and pulmonary homing of $CD4^+CD45RO^+$ helper memory T cells in cystic fibrosis

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Abstract Interstitial lung disease, although of prognostic impact for patients with cystic fibrosis (CF), remains difficult to assess without histopathologic investigations. As changes of peripheral blood lymphocyte subsets (LS) may accompany severe systemic lymphocyte immune responses, we compared peripheral LS of 44 patients with CF, 23 non-CF patients with recurrent pulmonary infections and 83 healthy controls (flow cytometry; CD3, CD19, CD16, CD56, CD4, CD8, CD11b, CD45RA, CD45RO, HLA-DR and CD25 antigens). Additional immunohistochemistry was performed on lung tissue of four CF patients aged 0·5, 12, 17 and 20 years, respectively. Patients with CF showed low absolute counts of $CD4^+CD45RO^+$ memory helper T cells, $CD16^+CD56^+$ NK cells, $CD8^+$ and interleukin-2 receptor-positive T cells in peripheral blood ($P < 0{\cdot}001$). Similar changes were registered in the non-CF patients with pulmonary infections, indicating that those were not specific for CF. Immunohistochemistry showed activation of bronchus-associated lymphoid tissue with interstitial accumulation of $CD4^+CD45RO^+$ T cells in the three older patients. Patients with CF show marked changes of peripheral blood LS which are presumably not CF-specific and may mirror homing to lung tissue in the course of interstitial lung disease. Further research should evaluate its usefulness in monitoring progression of lung disease in CF. © 2001 Elsevier Science Ltd

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Keywords cystic fibrosis; interstitial lung disease; memory T cells; immunophenotyping; bronchus-associated lymphoid tissue.

INTRODUCTION

Lung disease in cystic fibrosis (CF) is a multi-factorial process characterized by defective airway clearance of microorganisms, especially of *Pseudomonas aeruginosa*, due to impaired mucociliary clearance, altered function of the respiratory epithelium and, possibly, reduced innate immune defense. Simultaneously there is enhanced intrabronchial antimicrobial immune defense with neutrophils playing a central role: they fail to clear the airways of pathogens but, together with monocytes and airway epithelial cells, produce chemokines, exoenzymes and reactive oxygen specimens which enhance the inflammatory response and directly destroy lung tissue (1–3). Lymphocytes contribute to this process, too. Pulmonary interstitial T- and B cell infiltrations are present from early infancy, presumably leading to a Th2-type inflammatory response with increased interleukin-6 concentrations in bronchoalveolar lavage fluid, sputum, plasma and tracheal gland secretions, as well as increased synthesis of interleukin-4 and decreased synthesis of interferon-gamma by peripheral blood mononuclear cells (3–9). This interstitial inflammation may be related to allergic pulmonary complications, to hypergammaglobulinemia, to allergic bronchopulmonary aspergillosis, and to acute bronchiolitis obliterans organizing pneumonia, and may even shorten survival (8, 10–12).

Compared with neutrophils and the intrabronchial compartment, knowledge on the lymphocyte system and on interstitial pneumonia in CF is limited. On the one hand, immunophenotyping of lymphocyte subsets (LS) in lung tissue has been performed very rarely (5) and lymphocyte analysis in bronchoalveolar lavage fluid is limited by the abundance of neutrophils in bronchial secretions. On the other hand, peripheral blood lymphocyte phenotyping, which mirrors immune function in various inflammatory diseases, has revealed contradictory results, showing an increased or normal CD4/CD8 ratio and decreased or normal relative $CD4^+$ and $CD8^+$ cell counts (10,13–16). These latter studies, however, were confined to only few LS and to a small number of patients. We hypothesized that a more extensive

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analysis might demonstrate hitherto unknown changes of peripheral blood LS, characteristic of the inflammatory response in CF.

PATIENTS AND METHODS

Patients

Over a 5-year period we prospectively performed lymphocyte phenotyping from peripheral blood lymphocytes of 56 patients with CF (1 ml EDTA). For data analysis, patients not undergoing inhalative or systemic corticosteroid therapy and with no allergic bronchopulmonary aspergillosis or acute pulmonary inflammation were selected. Finally, data of 44 patients (age: 1 month –36 years, 12 males) were available for evaluation, and the most recent investigation of each patient was selected. The data were compared with those of 83 healthy controls (age: 1 month–29 years, 47 males) prospectively measured during the same period of time and of 23 infants and children routinely investigated in our outpatient clinic because of severe recurrent bronchopulmonary infections (age: 4 months–8 years, 14 males). The healthy controls belonged to the hospital staff or were attending the outpatient clinic for routine checkups. None had a history of recent or recurrent infections or allergic diseases, or was receiving acute or chronic medical treatment. Informed consent was given by all patients or parents. The study was approved by the Ethics Committee of the University.

Histopathology was performed on lung tissue from four patients with CF: a 6-month-old infant (patient 1) who had died from left heart failure in the course of acute bronchopneumonia, a 20-year-old patient with CF and bronchiolitis obliterans organizing pneumonia (BOOP) (patient 2), and two patients, aged 12 and 17 years, with right sided pneumonectomy because of life-threatening unilateral lung disease (patients 3 and 4). The clinical courses of patients 2, 3 and 4 have been described previously (11,17).

Clinical data of patients with cystic fibrosis

Sputum samples or deep throat swabs from children below 5 years of age were evaluated for the presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In patients aged 5 years or more, pulmonary function testing was performed without prior bronchospasmolysis according to the methods of Zapletal *et al*. (Bodytest II, E. Jaeger GmbH, Würzburg, Germany). The results were expressed as a percentage of predicted values (18).

Lymphocyte phenotyping

Surface antigens were stained by means of the whole blood technique recommended by Becton Dickinson Immunocytometry Systems (Heidelburg, Germany) who kindly provided antibodies for evaluation of normal values. In brief, 100 μl of whole blood were incubated with 20 μl antibody suspension (20 minutes, room temperature). After fixation and lysis (FACS^{TM} lysis solution, 10 min, room temperature) the white blood cells were washed (phosphate buffered saline, 10 min, 300 **g**) and resuspended in phosphate buffered saline. The antibody panel consisted of antibodies against common surface antigens of T (CD3), B (CD19), NK (CD16CD56), helper (CD4) T and suppressor/cytotoxic (CD8) T cells as well as against the T cell activation markers interleukin-2 receptor (CD25, IL-2R) and HLA-DR antigen (MHCII class). CD11b expression on $CD8^+$ T cells distinguishes suppressoric ($CD11b^+$) from cytotoxic ($CD11b^-$) cells (19). $CD4^+CD45RO^+$ cells are considered an activated and short-life memory helper T cell subset that expresses IL-2R, rapidly proliferates after antigen contact to effector T cells, and synthesizes cytokines. $CD4^+CD45RA^+$ cells comprise naive helper T cells and a subset of quiescent, long-life and slowly responding memory cells that develop from $CD4^+CD45RO^+$ cells in the absence of antigen contact (20). Antibody panels [FITC/PE (clone1/clone2)]: IgG1 isotype control/IgG2 isotype control, CD45/CD14 (2D1/MΦP9); CD3/CD19 (SK7/4G7); CD4/CD8 (SK3/SK1); CD3/HLA-DR (SK7/L243); CD3/CD25 (SK7/2A3); CD3/CD16CD56 (SK7/B73.1,MY31); CD8/CD11b (SK1/D12); CD45RA/CD4 (L48/SK3); CD45RO/CD4 (UCHL-1/SK3); (CD45RO, DAKO; all other antibodies: Becton Dickinson). Two-colour flow cytometry was performed using a FACScan flow cytometer calibrated with CaliBRITE beads and FACSComp software. For each test tube at least 15 000 white blood cells were analysed. For all patients and controls the sum of relative T ($CD3^+$), B ($CD19^+$) and NK ($CD16^+CD56^+$) lymphocyte counts was $100 \pm 5\%$. Automated data pre-analysis was performed by means of SimulSET^{TM} software, Attractor^{TM} software and quadrant statistics (FACScan flow cytometer and software, Becton Dickinson). Quadrants were defined according to the fluorescence characteristics of lymphocytes stained with fluorescence-conjugated, non-specific control antibodies. After automatic pre-analysis by means of attractor gating, visual control of all plots was performed. All values were expressed as percentages of gated lymphocytes. Absolute counts were recalculated from relative counts and absolute white blood cell counts, analysed by means of a Coulter MAXM$^{®}$ cell analyser.

Histopathology and immunohistochemistry

Haematoxylin–eosin, Elastica van Gieson and immunostaining were performed on 4 μm thick paraffin sections from lung tissue blocks. All tissue samples were immunostained in parallel, using a routine protocol for an indirect peroxidase method with DAB as chromogen. Sections from hyperplastic tonsils served as specimens

for negative or positive controls, respectively. The following antibodies (antibodies: DAKO Hamburg, Germany) were used [antigen specificity/clone (stained cell populations)]: CD68/M814 (monocytes), CD20/M755 (B-cells), CD4/MT310 (helper T cells, monocytes), CD8/M7103 (suppressoric/cytotoxic T cells), CD45RA/4KB5 (resting B cells, naive T cells), CD56/MOC-1 (NK cells), CD45RO/UCHL-1 (memory T cells).

Statistical analysis

Although pediatric lymphocyte subsets are age-dependent, for statistical analysis the data of all patients or control persons respectively were pooled, and an overall analysis was performed, with a significance level of $P < 0{\cdot}001$ being selected (two-tailed Mann–Whitney *U*-test, SPSS software). This procedure was not sensitive to subtle changes, but aimed at detecting strong and age-independent factors, reducing the experiment-wise type-I error rate in the presence of many dependent variables and a limited population size. Age-related changes of LS in CF were summarized only in a descriptive fashion, including only major LS and those where statistical overall analysis had revealed significant changes. Because the antibody panel was expanded during the study period, data on CD11b expression were available from 42 and on CD45RA/RO expression from 37 patients with CF only.

RESULTS

Clinical data of patients with cystic fibrosis

The high prevalence of *P. aeruginosa*-positive patients in the first age group mirrors the preselection of severely diseased patients treated at our hospital (Table 1). As expected, pulmonary function analysis revealed progression of lung disease with age.

Lymphocyte subsets in patients with CF

Comparison of patients and controls revealed no differences between absolute white blood cell counts, monocyte counts and neutrophil counts. Patients with CF, however, showed a marked lowering of $CD4^+CD45RO^+$ (memory helper) cells, of T cells expressing IL-2R, of $CD8^+$ cells and of $CD16^+56^+$ NK cells ($P < 0{\cdot}001$; Table 2). As can be hypothesized from Table 3, the lowering of distinct peripheral LS may already occur at a young age ($CD4^+CD45RO^+$) or progress with age ($CD8^+CD11b^+$; suppressoric).

Patients with CF and *P. aeruginosa* and/or *S. aureus* in sputum did not differ from the non-colonized patients with respect to their lymphocyte subsets.

Lymphocyte subsets in patients with bronchopulmonary infections

Compared to healthy controls, patients with recurrent bronchopulmonary infections showed similar changes to those registered in patients with CF; i.e. a decrease of $CD4^+CD45RO^+$ cells and of T cells expressing IL-2R ($P < 0{\cdot}001$; data not shown).

Morphological changes in lung tissue

Patient 1 showed acute bronchopneumonia with intra-alveolar granulocytes, no interstitial pneumonia, no lymphoid aggregates or follicles, and only very sparse lymphocytes. In patients 2, 3 and 4, lymphoid aggregates

Table 1. Clinical data of the patients

	age group 1 (n=6) 1 month–3 yrs	age group 2 (n=8) 4–9 yrs	age group 3 (n=12) 10–15 yrs	age group 4 (n=18) ≥ 16 years
FEV_1*	not done	91 (67–101)	73 (63–78)	53 (33–73)
FVC*	not done	90 (73–97)	71 (63–74)	66 (43–85)
Mean expiratory flow at 50% of vital capacity*	not done	74 (39–114)	56 (32–81)	25 (13–46)
Mean expiratory flow at 25% of vital capacity*	not done	44 (32–116)	38 (23–48)	19 (13–38)
TLC*	not done	100 (87–119)	92 (83–97)	96 (89–115)
Patient sputum *Pseudomonas aeruginosa*-positive	n=4 (66%)	n=3 (37·5%)	n=9 (75%)	n=13 (72·2%)
Patient sputum *Staphylococcus aureus*-positive	n=1 (17%)	n=2 (25%)	n=3 (25%)	n=5 (27·2%)

*Values expressed as median (25%–75% percentiles) (% of predicted value).
FEV: forced expiratory volume in 1 sec; FVC: forced vital capacity; TLC: total lung capacity.

Table 2. Comparison of lymphocyte subsets between patients with CF and controls

	All patients with CF age: 1 month–36 yrs (n=44)	All controls age: 1 month - 29 yrs (n=83)	Significant differences
Leucocytes $\times10^{-3}\mu l^{-1}$	6·3 (4·9–7·9)	6·7 (5·4–8·7)	
Lymphocytes $\times10^{-3}\mu l^{-1}$	1·9 (1·4–2·4)	2·5 (1·8–3·3)	
$CD3^+$ $\times10^{-3}\mu l^{-1}$	1·5 (1·1–1·8)	1·8 (1·3–2·6)	
% $CD3^+$ in lymph	76 (69–81)	69 (65–74)	P<0·001
%HLA-DR^+ in $CD3^+$	9 (6–11)	8 (6–12)	
%$CD25^+$ in $CD3^+$	11 (9–16)	15 (11–23)	
$CD3CD25^+$ $\times10^{-3}\mu l^{-1}$	0·19 (0·14–0·24)	0·3 (0·23–0·36)	P<0·001
$CD19^+$ $\times10^{-3}\mu l^{-1}$	0·28 (0·19–0·41)	0·38 (0·26–0·74)	
% $CD19^+$ in lymph	15 (12–18)	17 (13–21)	
%$CD16CD56^+$ in lymph	9 (6–13)	12 (9–15)	
$CD16CD56^+$ $\times10^{-3}\mu l^{-1}$	0·15 (0·11–0·27)	0·3 (0·23–0·39)	P<0·001
CD4/CD8 ratio	1·5 (1·1–2)	1·2 (1–1·5)	
$CD8^+$ $\times10^{-3}\mu l^{-1}$	0·6 (0·4–0·8)	0·9 (0·6–1·2)	P<0·001
%$CD8^+$ in lymph	29 (24–36)	33 (28–38)	
ratio $CD11b^-/CD11b^+$ in $CD8^+$	3·5 (2·3–5·9)	3 (2–4·4)	
$CD4^+$ $\times10^{-3}\mu l^{-1}$	0·9 (0·5–1·1)	1 (0·7–1·4)	
%$CD4^+$ in lymph	44 (38–50)	40 (35–46)	
$CD45RA^+CD4^+\times10^{-3}\mu l^{-1}$	0·6 (0·3–0·8)	0·7 (0·4–1·1)	
%$CD45RA^+$ in $CD4^+$	63 (56–73)	60 (51–69)	
$CD45RO^+CD4^+\times10^{-3}\mu l^{-1}$	0·4 (0·3–0·5)	0·7 (0·5–1)	P<0·001
%$CD45RO^+$ in $CD4^+$	49 (37–57)	57 (45–67)	

Values expressed as median (25%–75% quartiles).

Table 3. Age-related description of major lymphocyte subpopulations of patients with CF and controls

Age group	1 (1month–3yrs)	2 (4–9yrs)	3 (10–15yrs)	4 (⩾16yrs)
Patients with CF	n=6	n=8	n=12	n=18
$CD3^+$ $\times10^{-3}\mu l^{-1}$	2·9 (2·4–3·7)	1·6 (1·5–1·7)	1·4 (1·3–1·9)	1·2 (0·8–1·7)
% $CD3^+$ in lymph	76 (61–81)	74 (66–79)	77 (66–84)	76 (70–81)
$CD3CD25^+$ $\times10^{-3}\mu l^{-1}$	0·23 (0·16–0·3)	0·19 (0·14–0·22)	0·2 (0·12–0·22)	0·17 (0·13–0·24)
$CD4^+$ $\times10^{-3}\mu l^{-1}$	2·1 (1·7–2·2)	1 (0·9–1·1)	0·8 (0·6–1)	0·6 (0·4–0·9)
$CD45RO^+CD4^+\times10^{-3}\mu l^{-1}$	0·4 (0·3–0·6)	0·4 (0·4–0·5)	0·4 (0·3–0·6)	0·4 (0·3–0·5)
$CD8^+$ $\times10^{-3}\mu l^{-1}$	0·9 (0·6–1·9)	0·6 (0·5–0·7)	0·7 (0·4–0·8)	0·4 (0·4–0·6)
ratio $CD8^+11b^-/CD8^+CD11b^+$	2·9 (1·8–7)	3·1 (2·7–3·5)	4 (1·8–7·6)	4·7 (2·8–6·2)
$CD19^+$ $\times10^{-3}\mu l^{-1}$	0·4 (0·2–1·3)	0·4 (0·3–0·6)	0·3 (0·1–0·5)	0·2 (0·2–0·3)
$CD16CD56^+$ $\times10^{-3}\mu l^{-1}$	0·29 (0·13–1·1)	0·23 (0·14–0·27)	0·16 (0·11–0·27)	0·1 (0·14–0·26)
Controls	n=17	n=21	n=19	n=26
$CD3^+$ $\times10^{-3}\mu l^{-1}$	3·4 (2·8–4·6)	2 (1·5–2·6)	1·5 (1·2–1·9)	1·4 (1·1–1·7)
% $CD3^+$ in lymph	67 (63–74)	68 (65–73)	69 (68–74)	71 (66–77)
$CD3CD25^+$ $\times10^{-3}\mu l^{-1}$	0·27 (0·29–0·37)	0·29 (0·22–0·33)	0·23 (0·17–0·28)	0·38 (0·33–0·49)
$CD4^+$ $\times10^{-3}\mu l^{-1}$	2·4 (1·7–3·2)	1·1 (0·8–1·4)	0·8 (0·6–1)	0·9 (0·7–1·1)
$CD45RO^+CD4^+\times10^{-3}\mu l^{-1}$	1·1 (0·8–1·2)	0·8 (0·5–1)	0·5 (0·4–0·6)	0·7 (0·5–0·8)
$CD8^+$ $\times10^{-3}\mu l^{-1}$	1·2 (1–1·7)	0·9 (0·7–1·2)	0·8 (0·6–1)	0·7 (0·5–0·9)
ratio $CD8^+11b^-/CD8^+CD11b^+$	4·7 (2·7–6·6)	2·9 (1·9–4·5)	3·7 (2·8–4·4)	2·3 (1·6–2·9)
$CD19^+$ $\times10^{-3}\mu l^{-1}$	1·2 (1–1·9)	0·5 (0·3–0·7)	0·4 (0·3–0·5)	0·2 (0·2–0·3)
$CD16CD56^+$ $\times10^{-3}\mu l^{-1}$	0·36 (0·27–0·77)	0·32 (0·24–0·46)	0·28 (0·25–0·37)	0·25 (0·18–0·35)

Values expressed as median (25%–75% quartiles).

and follicles were prominent and located in the bronchial submucosa. As described previously, lung tissue of patient 2 showed the characteristics of BOOP (11). Lung tissue of patients 3 and 4 furthermore showed areas of abscess formation, areas of preserved alveolar architecture with or without alveolar septal thickening, and areas of fibrous scarring. Numerous foamy alveolar macrophages were found within alveoli. Macrophages

and neutrophils were abundant inside abscess formations but virtually absent from interstitial tissue. In accordance with the literature (21), three compartments of interstitial lymphocyte inflammation could be defined: alveolar septa, the bronchial subepithelial and submucous layer, and bronchus-associated lymphoid tissue (BALT) with lymphoid aggregates and lymphoid follicles; the latter consisting of the follicular area bounded by the parafollicular area and the dome area [Fig. 2(a), (b)]. Compared to patients 3 and 4, interstitial inflammation was more pronounced in patient 2 in whom abscess formation was absent.

In patient 1, the sparse and randomly distributed interstitial lymphocytes consisted of $CD45RA^+$- and $CD4^+$-T cells. $CD20^+$ B cells were virtually absent from interstitial tissue. Notable and randomly distributed interstitial $CD56^+$ cell infiltrations were found in this patient only, whereas $CD8^+$ and $CD45RO^+$ cells were not found. In patients 2, 3 and 4, T cells were most prominent in parafollicular areas, but were also found in follicular areas forming aggregates, in dome areas, in alveolar septa and in the bronchial subepithelium. The vast majority of these T cells were $CD4^+$ and $CD45RO^+$ [Fig. 2(a)]. Notable $CD8^+$ cell counts were found only in parafollicular areas and in the bronchial subepithelial layer of patient 2. $CD20^+$ B cells were dominant and randomly distributed in follicular areas. Less frequent $CD20^+$ B cell counts were found in the dome and parafollicular areas, and in the submucous tissue adjacent to lymphoid follicles. Rare $CD20^+$ B cell counts were also recorded in the bronchial subepithelium [Fig. 2(b)]. Marked plasma cell infiltrations ($CD20^-$) were found in the bronchial subepithelium. Plasma cells were surrounded by $CD4^+$ and $CD45RO^+$ T cells. $CD45RA^+$ cells (resting B cells, naive T cells) were found in follicular areas only.

DISCUSSION

This investigation is the first to demonstrate a marked decrease of peripheral $CD4^+CD45RO^+$ memory helper T cells and of NK cells in a larger group of patients with CF (Table 2). Additionally, considering the severe lung infection and the known increase of soluble Il-2R in CF serum (22), we found an astonishingly low expression of the activation marker IL-2R on T cells. Neutrophils and monocytes, in contrast, were not affected in a comparable fashion, although these LS dominate the intrabronchial inflammation. Hypothetically, shedding of CD4 and CD8 surface antigens by neutrophil exoenzymes might decrease surface antigen density, impair flow-cytometric detection and mimic a decrease of distinct LS. However, in patients with CF, shedding has been shown to be restricted to the airway lumen (16); to exclude this bias, only patients in a stable clinical condition were enrolled. CD4 shedding should also have affected $CD4^+CD45RA^+$ cell counts, which were normal, and histopathology revealed a high submucous $CD4^+$ cell density where CD4 shedding should have been most extensive. Therefore, the observed findings can be assumed to indicate a specific decrease of distinct peripheral LS.

One cause for this decrease might be a suppression of the lymphocyte system due to the extensive intrabronchial neutrophil inflammation, a hypothesis controversially discussed in the literature. On the one hand, investigation of peripheral blood lymphocytes revealed a depressed *in-vitro* B and T cell response to mitogen stimulation and a reduced T cell cytotoxicity (6,23–26). These findings were more severe in patients with advanced lung disease and agree with the observed peripheral lowering of activated lymphocytes. On the other hand, hypergammaglobulinemia with high anti-

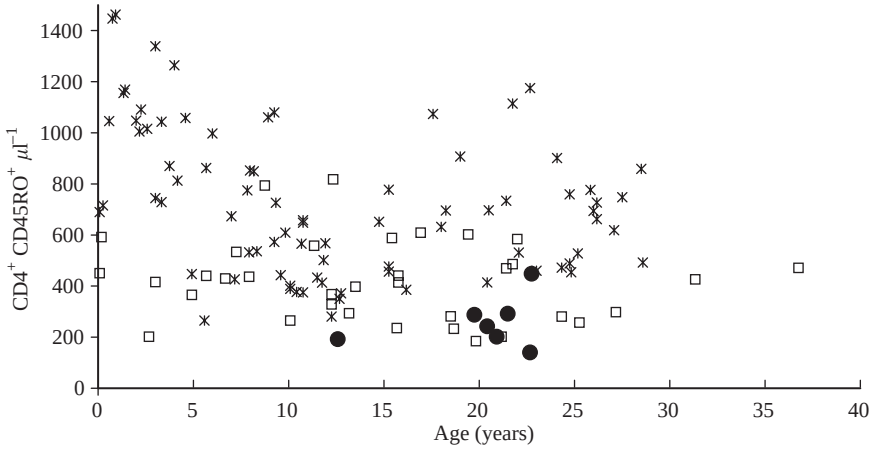

Fig. 1. Decrease of memory helper T cells ($CD4^+CD45RO^+$) in 83 controls (*), in the three older patients where immunohistochemistry of lung tissue was performed (●, several measurements per patient), and in the remaining 41 patients with CF (□). In patients with CF, absolute counts of $CD45RO^+$ helper T cells are low. A similar lowering was observed for $IL\text{-}2R^+$ T cells, whereas absolute counts of $CD4^+CD45RA^+$ (naïve helper) T cells were normal (Table 2).

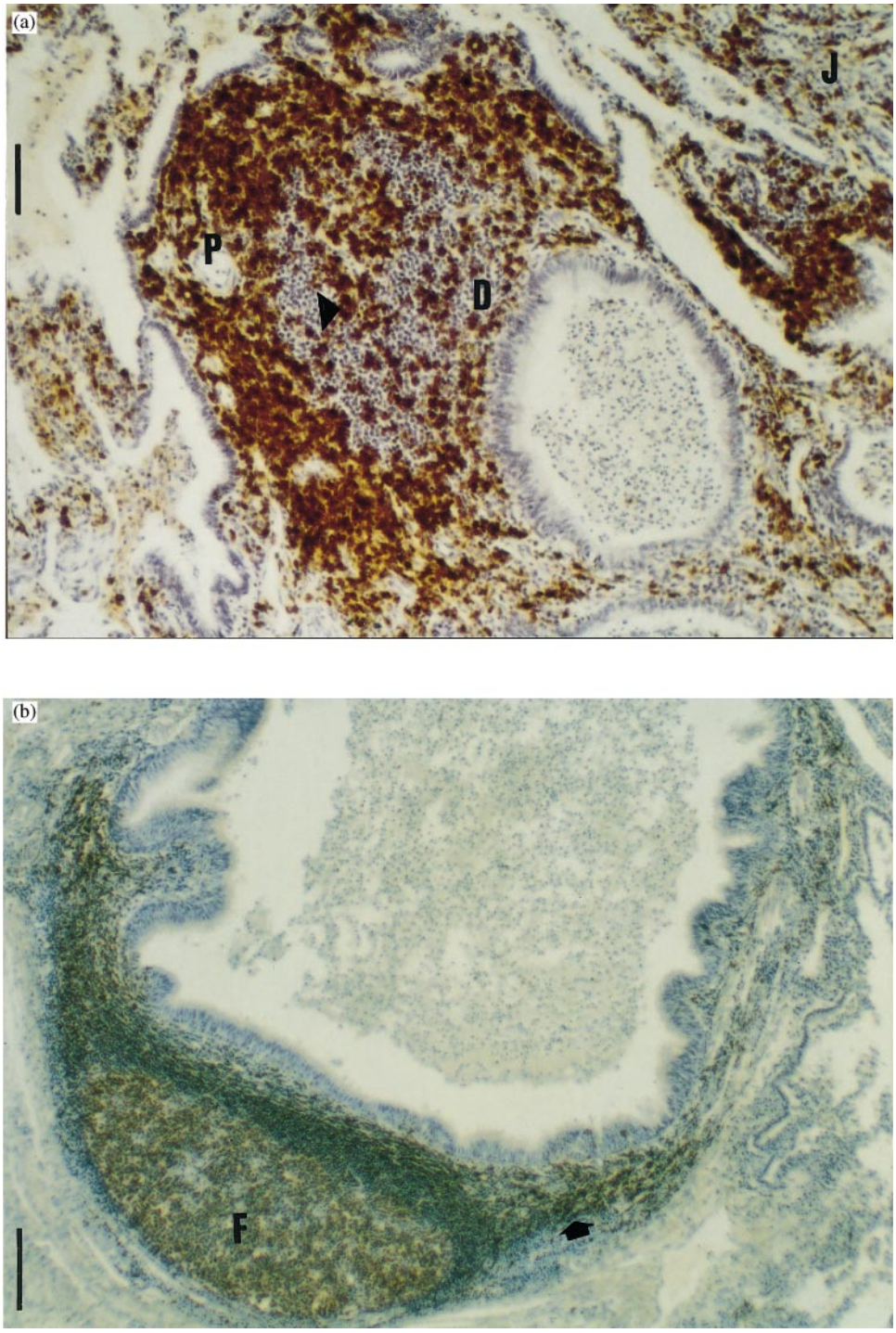

Fig. 2. (a) Lung tissue stained for $CD45RO^+$ T cells. Marked accumulation of $CD45RO^+$ T cells in the parafollicular area (P), in the dome area (D) and in interstitial tissue (J). $CD45RO^+$ T cell aggregates are present in follicular areas (arrow). Bar: 150 μm. (b) Lung tissue stained for $CD20^+$ B cells. $CD20^+$ B cells are found predominantly in follicular areas (F), but are also present in parafollicular areas, in the dome areas, in the submucous tissue adjacent to lymphoid follicles (arrow) and, to a much lesser extent, in the subepithelial layer. Bar: 200 μm.

body titres to *P. aeruginosa* exoenzymes is characteristic of CF (1–3), and immunosuppressive treatment with ibuprofen or steroids improves lung function of patients with CF (27).

Homing to pulmonary tissue may be a further explanation for the decrease of distinct peripheral LS. This hypothesis is supported by our histopathologic findings, showing $CD4^+CD45RO^+$ T cells aggregated within lym-

phoid follicles, scattered throughout the interstitial spaces and surrounding submucous plasma cells in the three older patients [Fig. 2(a), (b)]. As $CD45RO^+$ T cells account for the majority of peripheral IL-$2R^+$ T cells (28), pulmonary pooling of this LS would also explain the peripheral decrease of IL-$2R^+$ T cells. However, due to the limited number of tissue specimens available and the preselection of our patients, this hypothesis remains to be verified in the course of further, more extensive studies. Moreover, $CD8^+$ and NK cells, that were low in peripheral blood, were not increased in lung tissue although these LS may be important for immune defense (29,30) against *P. aeruginosa.* Possibly, $CD8^+$ cells migrate to the airway lumen where they are lost during immune defense or become undetectable after CD8 antigen cleavage (16). NK and $CD8^+$ cells might also be more frequent in lung tissue of patients with less severe bacterial inflammation: NK cells, for instance, were present in patient 1 where chronic lung disease was absent, and $CD8^+$ cells were present in the patient with bronchiolitis obliterans organizing pneumonia (patient 2). Interstitial $CD8^+$ cells have also been described in lung tissue of a patient with allergic bronchopulmonary aspergillosis and CF (5).

Neither the decrease of peripheral blood LS nor the observed interstitial lymphocyte distribution, however, can be considered CF-specific. Although lung disease had been present for a short period of time only, our non-CF patients with recurrent bronchopulmonary infections showed a decrease of peripheral $CD4^+CD45RO^+$ and of IL-$2R^+$ T cells. Moreover, the immunohistochemical findings of the cases reported here share the typical characteristics of activated BALT, which is not observed in healthy adults (31), but is known from different inflammatory pulmonary diseases such as asthma, sarcoidosis or chronic hypersensitivity pneumonitis (13,14,21). In patients with sarcoidosis and asthma, even a secondary decrease of peripheral $CD4^+$ and $CD8^+$ cells may occur (13,14). In patients with chronic hypersensitivity pneumonitis, where interstitial $CD8^+$ cell accumulation is not found either (21), repeated exposure to organic inhalation antigens leads to similar complications as known from patients with CF; i.e. persistent small airway inflammation, interstitial fibrosis and bronchiolitis obliterans organizing pneumonia. In a comparable fashion, ongoing intrabronchial *Pseudomonas* antigen challenge may stimulate cytokine synthesis in CF bronchial epithelial and intrabronchial inflammatory cells, establish a Th2-type cytokine milieu, initiate and maintain the interstitial accumulation and peripheral decrease of highly activated lymphocytes, and contribute to pulmonary complications (10,11). *Pseudomonas* exoenzyme S may even directly activate $CD4^+$ cells (32) and enhance this process. Considering these mechanisms and the high impact of *P. aeruginosa* for lung disease in CF, it seems astonishing that patients with or without *Pseudomonas* colonization did not differ with respect to their peripheral LS. However, the absence of *P. aeruginosa* from sputum samples does not rule out its presence from small airways, and further factors that have not been taken into account in the present study, such as viral pulmonary infections, may also influence the inflammatory process (33).

Further studies on larger patient populations and numbers of lung tissue specimens are needed to specify the relationship between changes of distinct peripheral blood lymphocyte subsets, pulmonary infection and interstitial pneumonia. These studies should also investigate whether the decrease of distinct peripheral blood LS progresses with the age of the patients and the decline of pulmonary function. Possibly, immunophenotyping of peripheral blood LS will become a useful indirect tool for assessment of interstitial pulmonary inflammation in CF.

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